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Inhibition of HMG-CoA reductase and lipid peroxidation in the rats liver by selected zingiberaceae

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ABSTRACT

Cardiovascular disease is the leading cause of death worldwide. One of the major risk factors for cardiovascular disease is hyperlipidemia. This study aimed to determine the potential of Zingiberaceae (10 species) as inhibitor of HMG-CoA reductase enzyme activity and lipid peroxidation. This study was conducted by 2 methods including assay of HMG-CoA reductase inhibition and lipid peroxidation test. The study was performed by in vitro method, using 20% rat liver homogenate. The inhibition of HMG-CoA reductase enzyme was done by reacting liver homogenate, HMG-CoA substrate, which added the Zingiberaceae extract compared with simvastatin as standard drug. The absorbance of the mixture was measured by a Microlab 300 spectrophotometer at a 340 nm wavelength. Lipid peroxidation assay was induced by the $FeSO_4.7H_2O$ solution. The absorbance value measured using a spectrophotometer at a 532 nm wavelength. Lipid peroxidation inhibition was characterized by absorbance of the test extract, compared with the control group. The obtained data was calculated as percent of inhibition and was used to calculate IC_{50} extract test. The results showed that the 10 ethanolic extracts of Zingiberaceae rhizomes have activity as inhibitor HMG-CoA reductase enzyme with IC_{50} value range 65.8±4.1 – 203.3±15.2 ppm and IC_{50} for simvastatin as standard drug was 6.8±0.1 ppm. Inhibition of lipid peroxidation with IC_{50} value range 13.5±5.0 – 219.6±4.3 ppm and simvastatin as standard drug was 1.8±0.5 ppm. This study can be concluded that the Zingiberaceae rhizomes have potential role as antihyperlipidemic agents through inhibition of HMG-CoA reductase enzyme activity and preventing lipid peroxidation.

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Keywords: hyperlipidemia, HMG-CoA reductase inhibitor, lipid peroxidation, zingiberaceae

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INTRODUCTION

Cardiovascular disease is the leading cause of death worldwide. The World Health Organization (WHO) reports that hyperlipidemia is a major risk factor for cardiovascular disease and atherosclerosis. About 31% of deaths caused by cardiovascular disease include coronary heart disease and stroke (Mendis, Puska, Norrving, & Organization, 2011). The initial stage of coronary heart disease (CHD) is hyperlipidemia. Hyperlipidemia is a metabolic disorder indicated by an increase in total cholesterol, low-density lipoprotein (LDL), or triglycerides, and a decrease in high-density lipoprotein (HDL), or combination of both in the blood (Wells, DiPiro, Schwinghammer, & DiPiro, 2014). It has been found that there is an inverse relationship between HDL cholesterol levels and coronary heart disease risk (Rader & Hovingh, 2014). Moreover, the oxidized LDL lipoprotein (oxLDL) or lipid peroxidation in hyperlipidemia plays an important role in atherogenesis by increasing the lipid deposit in the arterial wall (Moriel *et al.*, 2000). The presence of oxLDL is a biomarker of cardiovascular disease (Trpkovic *et al*., 2015).

There is a relationship between the prevalence of hyperlipidemia and hypertension. Hypertension and hypertriglyceridemia are important components of the metabolic syndrome. Therefore, the increasing prevalence of these components leads to metabolic disease. Moreover, the relationship between blood pressure, central obesity, and the high levels of insulin, not only observed in adults, but has also been detected in children and adolescents. The increased prevalence of hyperlipidemia will be associated with the increasing prevalence of hypertension, result in the increase cardiovascular risk factors. Thus, cardiovascular disease can be prevented by preventing the main risk factor. (Bhalavi, Deshmukh, Atram, & Mahajan, 2014).

HMG-CoA reductase is an enzyme that plays a role in controlling the biosynthesis of endogenous cholesterol in the liver. HMG-CoA reductase catalyzes HMG-CoA conversion into mevalonate, as a sterol precursor including cholesterol. The pathogenesis of hyperlipidemia may be due to hyperactivity of the HMG-CoA reductase enzyme. This enzyme is a target of statin drugs, namely the class of HMG-CoA reductase inhibitors. Statins work through inhibition of endogenous cholesterol biosynthesis in the liver thus increasing LDL receptor activity in the liver thereby increasing the clearance of LDL cholesterol in plasma. In addition, statins can also decrease LDL production by decreasing the production of very low-density lipoprotein (VLDL) in the liver. Statins as first-line hyperlipidemic drugs not only improve lipid profile but also improve vascular inflammation as the initial process of atherosclerosis (Blum, 2014). Therefore, drug research that can inhibit the enzyme HMG-CoA reductase is very potential to be developed, especially from herbal medicines.

The prevalence of hyperlipidemia is still high and increasing every year, especially in Indonesia, indicating that the existing treatment has not been able to control the disease. It is an opportunity to find a safer and more effective drug in treating hyperlipidemia, using Indonesian herbal medicine. Family Zingiberaceae has been used in Indonesia to overcome various diseases including hyperlipidemia.

The Zingiberaceae family has held an important place for hundreds of years. Because of infusions and tinctures from these plants and other aromatic species have been used and are still used today as a component of herbal medicine to treat a variety of diseases (Tripathi & Singh, 2015). Zingiberaceae is a widely studied plant family and a potential source of medicinal plants. Various studies have shown that *Zingiber officinale* var rubrum has the potential to lower cholesterol, lower blood glucose levels and antioxidants (Srinivasan, 2017). Previous research has shown that Zingiberaceae can inhibit the alpha-glucosidase enzyme (Hasimun *et al*., 2016). This study aimed to determine the potential role of Zingiberaceae family (10 species) on the activity of HMG-CoA reductase enzyme. So the results of this study can optimize the use of Zingiberaceae family as a blood cholesterol-lowering agent.

MATERIALS AND METHODS

Plant materials

The study conducted using 10 species of Zingiberaceae namely: *Zingiber littorale* Nor., *Zingiber aromaticum* Val., *Zingiber zerumbet* (L) J.E.Smith., *Zingiber cassumunar* Roxb., *Zingiber ottensi* Val, *Zingiber officinale* Var *officinarum*, *Zingiber officinale* Var. Amarum., *Zingiber officinale* Var. Rubrum., *Kaempferia galanga* L., *Alpinia galanga* (L) Sw.This research uses rhizome plant parts. Zingiberaceae rhizome obtained from Manoko Lembang, Bandung, West Java. Plants have been carried out botanical identification in the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Bandung, West Java, Indonesia.

Preparation extract

The dried *Zingiberaceae* rhizomes were extracted using 96% ethanol solvent (purchased from Brataco, Bandung, Indonesia) by maceration method for 3 days. The filtrate was filtered, and concentrated using rotary evaporator at 60 °C and dried over a water bath at 60 ° C.

Phytochemical screening

Phytochemical screening was performed on ethanol extract of 10 *Zingiberaceae* rhizomes. Phytochemical screening aimed to identify classes of secondary metabolite compounds contained in *Zingiberaceae* rhizomes such as alkaloids, flavonoids, tannins, saponins, steroids.

Experimental design

Three Wistar 2-month-old male rats weighing 200-250 grams obtained from D'Wistar (Majalaya, Bandung, West Java, Indonesia). Test animals were acclimatized for 7 days in a cage with standard feed and drinking water, as well as 12 light dark duty cycles maintained. All treatments performed on test animals during the study were approved by the Ethical Committee of the Faculty of Medicine, Padjadjaran University, Bandung, West Java, Indonesia (No: 26/ UN6.C.10/PN/ 2018).

After acclimatization, the rats were sacrificed and the liver organ were isolated to make 20% liver homogenate in buffer phosphate pH 7.4.

HMG-CoA reductase enzyme activity assays

Prior to the test, a preliminary test of optimization of HMG-CoA substrate concentration was required to react with HMG-CoA reductase enzyme in liver homogenate 20%. The preliminary test was conducted to determine the optimum substrate concentration to react with HMG-CoA reductase enzyme. The results of the optimization obtained the substrate concentration required to activate HMG-CoA reductase enzyme in a homogenate of 20% was 0.1 ppm. Then the optimum concentration of HMG-CoA substrate was used to test enzyme activity in 20% homogenate of rat liver incubated with *Zingiberaceae* extract.

The test was performed by making the homogenate of the liver isolated from the liver of the test animal. The isolated liver was cleansed by 0.9% NaCl. The cleansed and weighed liver was then cut into small pieces, and weighed to make 20% homogenate in phosphate buffer pH 7.4 and centrifuged at 8000 rpm, to form supernatant and residue. The supernatant was separated from the residue as a sample (homogenate).

A 50 µL homogenate of 20% liver was added with 260 µL phosphate buffer pH 7.4, 42 µL Na2EDTA (purchased from Bratachem) 50 mM, 60 µL Dithiothreitol ((purchased from Sigma) 100 mM, 50 µL KCl 2,16 M, 12 µL substrate HMG-CoA (purchased from Sigma). The mixture was vortex for 10 secs and incubated for 30 minutes (at 37°C). then added 55 NADPH (purchased from Sigma) 1,105 mM and vortex for 10 secs and then incubation for 30 min (at 37^{o}C) (Table I). The incubation process is carried out to ensure the reaction between the substrate and the enzyme HMG-CoA reductase as well as the test extract. The reaction will produce color with different intensity, the absorbance of the mixture was measured by a Microlab 300 spectrophotometer at a 340 nm wavelength. The presence of inhibition of HMG-CoA reductase enzyme activity was characterized by decreasing sample absorbance value.

Lipid peroxidation assays

The lipid peroxidation activity test follows Selvam *et al*. (1987) with slight modifications. Lipid peroxidation was induced by the FeSO4.7H2O solution. The short test procedure was as follows: 2.2 mL of phosphate buffer pH 7.4, 0.2 mL FeSO4.7H2O 10 mM and 0.6 mL of liver homogenate were incubated in a mechanical shaker incubator at 37 ° C for 60 min. Added 0.5 mL of 40% trichloroacetic acid, 0.25 mL HCl 5 N and 0.5 mL 2% thiobarbituric acid. The mixture was stirred and then incubated in a water bath at 100 ° C for 10 minutes. The tube was cooled, then added 3 ml of chloroform and stirred. All test tubes were centrifuged at 2500 rpm for 10 minutes. The organic layer was separated (bottom). The supernatant absorbance was measured by spectrophotometer at 532 nm wavelength (Selvam & Kurien, 1987).

Data Analysis

The data obtained were shown as percent of inhibition. Increased percentage inhibition is proportional to the potential of the test extract as an inhibitor. Data was shown as the average value and standard deviation (n = 3). The IC50 was obtained by calculation of the percent value of inhibition at 3 different concentrations.

RESULT AND DISCUSSION

The extract yield of 10 *Zingiberaceae* rhizomes was obtained by maceration using 96% ethanol solvent for 3 days presented in Table I. The results of phytochemical screening of dried rhizomes are shown in Table II.

Table I. The yield of rhizome extracts of 10 species from the *Zingiberaceae* family (macerated by 96% ethanol)

Zingiberaceae	Yield (%)
Alpinia galanga (L) Sw.	1.6
Kaempferia galanga L.	1.7
Zingiber littorale Nor.	1.8
Zingiber zerumbet (L) J.E.Smith.	1.9
Zingiber officinale Var. Amarum	2.5
Zingiber officinale Var. Officinarum	0.8
Zingiber officinale Var. Rubrum	2.2
Zingiber aromaticum Val.	1.2
Zingiber ottensi Val.	2.8
Zingiber cassumunar Roxb.	2.9

Table II. Phytochemistry compounds from 10 species of Zingiberaceae family

Zingiberaceae	Content of				
	Alkaloid	**Flavonoid**	**Saponin**	**Steroid**	**Tanin**
Alpinia galanga (L) Sw.	−	−	−	−	+
Kaempferia galanga L.	−	−	−	+	+
Zingiber aromaticum Val.	−	+	+	−	+
Zingiber cassmunar Roxb.	−	+	−	−	+
Zingiber littorale Nor.	+	−	+	−	+
Zingiber officinale Var. Amarum	−	+	−	+	−
Zingiber officinale Var. Officinarum	+	+	−	−	+
Zingiber officinale Var. Rubrum.	+	+	−	−	+
Zingiber ottensi Val	−	+	−	+	+
Zingiber zerumbet (L) J.E.Smith.	−	+	+	+	−

(+): present , (-): absent

Zingiberaceae rhizomes as inhibitor HMG-CoA Reductase

The addition of Zingiberaceae extract to rat liver homogenate decreases the activity of HMG-CoA reductase enzyme indicated by decreased absorbance value of NADPH. This occurs because of inhibition of HMG-CoA reductase enzyme activity by Zingiberaceae extract where this enzyme activity always requires NADPH as a cofactor. NADPH undergoes an oxidation-reduction reaction that will be converted to $NADP^+$ and produces a color reaction when the active HMG-CoA reductase enzyme converts HMG-CoA into mevalonic acid and then converted to cholesterol.

The percent of inhibition obtained from the absorbance value was used to calculate the IC_{50} value of the Zingiberaceae extract. IC_{50} value shows the amount of Zingiberaceae extract concentration that can inhibit the activity of HMG-CoA Reductase enzyme by 50 percent. The smaller concentrations of IC_{50} indicate that the stronger activity of extract as an HMG-CoA reductase enzyme inhibitor (Table III).

Table III. IC_{50} concentration of Zingiberaceae rhizome as HMG-CoA reductase inhibitor

Zingiberaceae	IC_{50} (ppm)
Simvastatin	6.8 ± 0.1
Zingiber officinale Var. Rubrum	65.8 ± 4.1
Zingiber littorale Nor.	73.0 ± 8.7
Zingiber cassumunar Roxb.	73.6 ± 8.7
Zingiber ottensi Val.	74.1 ± 6.0
Zingiber officinale Var. Officinarum	74.2 ± 13.6
Zingiber officinale Var. Amarum	76.7 ± 2.8
Zingiber aromaticum Val.	88.1 ± 2.2
Zingiber zerumbet (L) J.E.Smith.	89.1± 5.3
Alpinia galanga (L) Sw.	199.6 ± 6.5
Kaempferia galanga L.	203.3 ± 15.2

Values are expressed as mean and error bar (n=3).
IC50 obtained from regression line of percentage inhibition.

Previous studies have reported that extract of *Zingiber officinale* Rosc. decreased liver enzyme activity such as AST, ALT, ALP and LDH, which increases due to iron accumulation in the liver (Gholampour, Ghiasabadi, Owji, & Vatanparast, 2017). Other studies have reported that the ethanol extract leaves *Zingiber officinale* Rosc has potent antioxidant activity (Tohma *et al*., 2017). Clinical studies in patients with type 2 diabetes mellitus, supplementation of *Zingiber officinale* improves glycemic control of patients. Its therapeutic effects occur through various mechanisms including inhibiting lipid peroxidation (Shidfar *et al*., 2015). Therefore, supplementation of *Zingiber officinale* may reduce the risk of chronic complications of diabetes (Khandouzi *et al*., 2015).

This study showed that the most potent inhibition potential of Zingiberaceae extracts against HMG-CoA reductase and lipid peroxidation activity are *Zingiber officinale* Var. Rubrum. and *Zingiber littorale* Nor. The effect of red ginger (*Zingiber officinale* Var. Rubrum) as an inhibitor of HMG-CoA reductase supports the results of previous studies which reported that red ginger has antihyperlipidemic activity capable of reducing total cholesterol and triglyceride levels and increasing HDL levels (Safitri, Kurniati, Adharani, Suciyati, & Adnyana, 2016). *Zingiber littorale* is one of 3 types of *Zingiber zerumbet* variants. The activity of *Zingiber littorale* as an inhibitor of HMG-CoA reductase enzyme, was first reported in this study. The results of previous studies showed that zerumbone, a sesquiterpene of *Zingiber zerumbet* Smith, was reported as an active compound that plays an important role in the inhibitory effect of the HMG-CoA reductase enzyme (Tzeng, Lu, Liou, Chang, & Liu, 2014)

CONCLUSION

Based on the results of this study can be concluded that Zingiberaceae rhizome ethanol extract has antihyperlipidemic activity through the mechanism of inhibition of liver enzyme HMG-CoA reductase and prevent lipid peroxidation therefore it may have potential activitiy as anti-hyperlipidemia and anti-atherosclerosis.

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